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PROCEEDINGS

Nutritional quality of human milk from Mediterranean lactating women: a preliminary approach towards personalised nutrition

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Introduction

Early life nutrition plays a key role in the future adult health. Human milk, the only natural source of food for the newborn, is in this way a food of predilection and should ensure an adequate metabolic programming. Indeed, breastfeeding has been shown to be beneficial for growth, development of neurological and cognitive functions, and prevention of some diseases in adulthood. These beneficial effects might be due to the presence in milk of polyunsaturated fatty acids, especially arachidonic and docosahexaenoic acids, that are essential for good development of the newborn [1] and have a biological imprinting on the expression of various enzymes involved in lipid metabolism [2]. These effects should also be linked to the presence of various proteins (insulin, leptin, bile salt-stimulated lipase, apolipoproteins, etc.).

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Objectives

The aim of this preliminary study was to determine, through lipidomic and proteomic approaches, if the nutritional quality of the milk from lactating women living in two different mediterranean localizations (Marseille and the Balearic Islands) was optimal.

Materials and methods

Human milk samples (colostrum, transitional and mature types) were collected in lactating women from Marseille (n = 22) and from the Balearic Islands (n = 4). Regarding the lipidomic approach, several physico-chemical characteristics of milk were determined such as lipid droplet size (laser light scattering, Microplus Mastersizer, Malvern), total lipid content (commercial kits or phosphorus quantitation), and fatty acid profile of triglycerides and phospholipids (gas chromatography). Concerning the proteomic approach, we focused on the measurement of bile saltstimulated lipase activity using a radiolabeled lipid emulsion, and we researched the presence of different known and new proteins using specific antibodies (one dimensional electrophoresis and western blotting, immunoassays).

Results and discussion

Our data show huge variations in the quantity and quality of the lipids and proteins secreted in human milk samples.

Lipidomic approach

We found that the majority of the milk samples do not reach the optimal levels recommended for essential fatty acids by

Table 1 Fatty acid profile of human milk total lipids (%)

Milk samples	LA	ARA	ALA	DHA
1	10.86	1.00	*0.44*	1.15
2	14.18	0.73	*0.46*	**0.59**
3	13.18	*0.35*	*0.79*	*0.00*
4	*7.87*	0.75	*0.38*	0.73
8	*7.67*	0.68	*0.38*	0.68
9	12.93	0.64	1.80	1.36
10	22.62	*0.32*	3.04	*0.23*
11	10.44	*0.09*	*0.72*	**0.57**
12	*8.51*	*0.00*	*0.44*	**0.61**
13	*7.47*	*0.37*	*0.63*	*0.21*
14	21.93	1.04	*0.42*	0.79
15	14.53	0.68	*0.57*	**0.59**
16	14.17	0.50	*0.50*	*0.25*
17	*8.80*	0.66	*0.32*	**0.65**
18	10.58	0.54	*0.51*	**0.53**
19	*7.48*	*0.21*	*0.47*	*0.09*
20	11.17	0.84	*0.87*	0.84
21	10.17	0.58	*0.73*	**0.61**
22	13.08	0.52	*0.76*	**0.44**
23	12.51	*0.29*	*0.46*	*0.36*
24	15.13	*0.31*	*0.64*	*0.00*
25	22.05	*0.34*	1.24	*0.01*
26	13.45	*0.27*	*0.57*	*0.01*

Fatty acid profile of total lipids was determined by gas chromatography. Milk samples were collected in lactating women from Marseille (milk samples 1–22) or from the Balearic Islands (milk samples 23–26). Levels of linoleic acid (LA), arachidonic acid (ARA), alpha-linolenic acid (ALA) and docosahexaenoic acid (DHA) are expressed in percentage of total lipids levels; levels out of recommendations for essential fatty acids by ESPGAN [3] are in *Italics*; levels out of recommendations for essential fatty acids by Gibson and Makrides [4] are in *bold*

ESPGAN (linoleic acid (LA) = 9–22%; alpha-linolenic acid (ALA) = 1–3%; arachidonic acid (ARA) = 0.4–1%; docosahexaenoic acid (DHA) = 0.4–1%) [3] or by Gibson and Makrides (DHA = 0.7–1%) [4] (Table 1). This has to be improved via specific dietary supplementation to the mothers. We also found that milk samples highly differ in

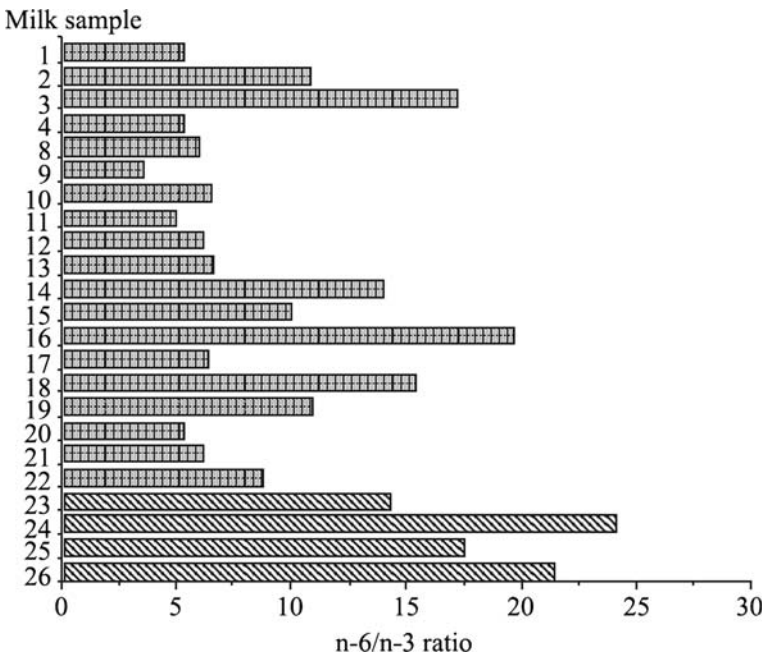
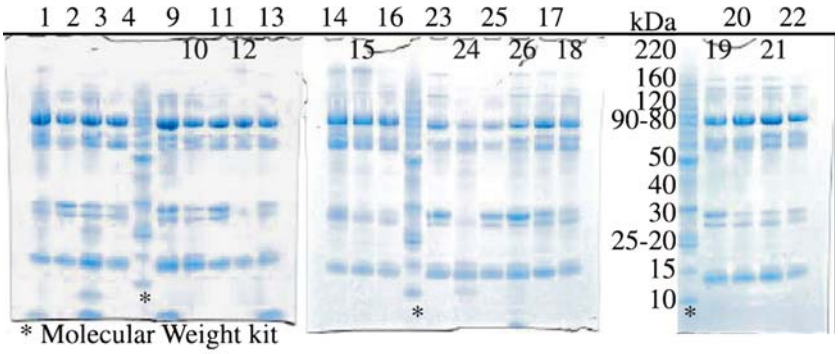


Fig. 1 Human milk n-6/n-3 ratios. The n-6/n-3 ratios were calculated using the sum of the total fatty acids belonging to the two families. Milk samples were collected in lactating women from Marseille (milk samples 1–22) or from the Balearic Islands (milk samples 23–26)



Milk sample	1	2	3	4	9	10	11	12	13	14	15
Proteins (g/100mL)	2,8	2,8	0,8	2,4	1,4	1,9	3,7	3,7	2,4	3,7	3,9

Milk sample	16	23	24	25	26	17	18	19	20	21	22
Proteins (g/100mL)	2.7	1.4	1.4	1.6	1.5	3.7	2.0	2.1	2.5	2.2	3.6

Fig. 2 SDS-PAGE (4–12% gradient) of the human milk samples. Milk samples were collected in lactating women from Marseille (milk samples 1–22) or from the Balearic Islands (milk samples 23–26). The volume of sample applied to the gel was calculated in order to run 15 µg total protein per samples

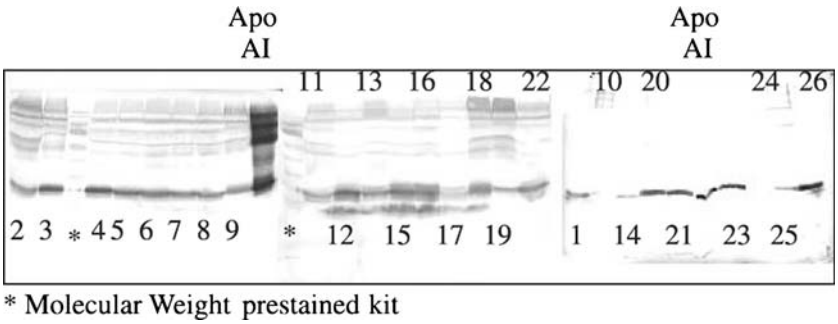


Fig. 3 Detection of Apo AI by Western blotting. Milk samples were collected in lactating women from Marseille (milk samples 1–22) or from the Balearic Islands (milk samples 23–26). The volume of sample used was 10 µL

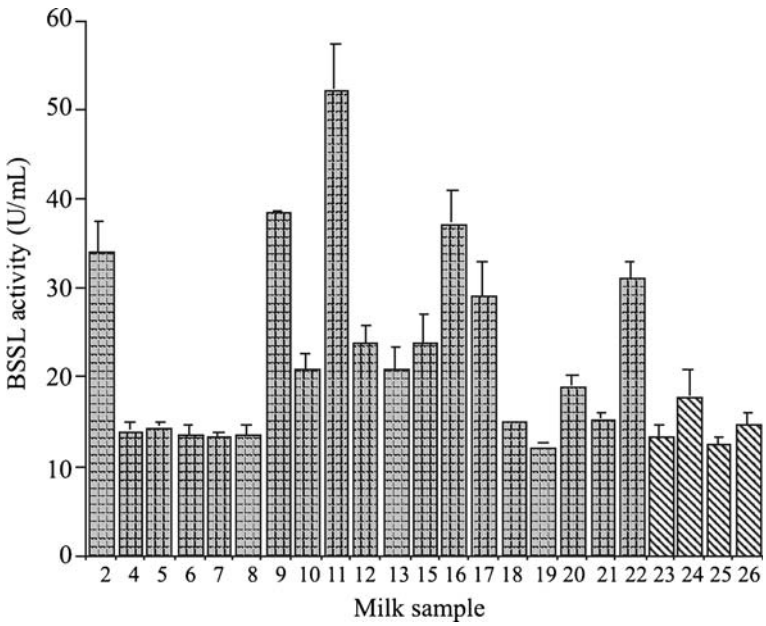


Fig. 4 Bile salt-stimulated lipase activity (U/mL). Bile salt-stimulated lipase activity was measured according to the original method of Hernell and Olivecrona [5], adapted by Freed [6]. Activities are expressed in Unit per milliliter (one lipase Unit correponds to the release of one µmol of fatty acid per minute). Milk samples were collected in lactating women from Marseille (milk samples 1–22) or from the Balearic Islands (milk samples 23–26)

their total n-6/n-3 ratios, ranging from 3 to 24 (Fig. 1). About 43% of the samples have a ratio from 5 to 6 in accordance with the recommendations [3], but 52% of them exhibit a ratio from 10 to 20 or more, especially for those collected in the Balearic Islands. This last point is worrying because it has been suggested that a too high dietary intake of n-6 fatty acids, to the detriment of n-3 fatty acids, improves adipose tissue development [5]. This could be linked to a too high linoleic acid and/or a too low docosahexaenoic acid consumption of the mother.

Proteomic approach

The protein contents and profiles are quite different among milk samples (Fig. 2). There is no evident link to the fact that milk is from preterm or term delivered mothers or belongs to colostrum (4, 8, 15), transitional (1, 2, 11, 12, 14, 17, 20) or mature type (3, 9, 13, 16, 18, 19, 21, 23, 24, 25, 26, 22). Protein profiles seem to be much different in milk samples from the Balearic Islands. As an example of the proteins we have detected by Western blotting, Apo AI (a small 28 kDa protein invoved in cholesterol transport and absorption) is present in all milk samples but at very different levels (Fig. 3). A quantitation using Western blotting and pure Apo AI calibration curve gave quantities ranging from 1 to 12 mg/L when present. A more sensitive quantification by ELISA is in progress in order to point out if this protein can be a biomarker for milk quality. We also measured bile salt-stimulated lipase activity (Fig. 4), and we found that it ranges from 12 to 52 U/mL (with an average of 21.8 ± 2.2 U/mL) in accordance with literature [5–7], and that milk samples from Balearic Islands seem to exhibit the lowest lipase activities. Bile salt-stimulated lipase plays a key role in intestinal lipid digestion in term and preterm infants, so it is of high interest to understand what causes the differences in the activity of this enzyme among milk samples.

Conclusion and perspectives

All this data indicate that human milk samples are not equivalent in terms of lipid and protein contents and profiles. Further studies will be conducted in order to specify the causes of the individual variations in milk quality by targeting the nutritional and genetic factors of the mother, with the final objective of proposing a personalised nutrition to lactating women.

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